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Electrochemical Investigation of Carbon Nanotube Modified Surfaces Based on Ferricyanide and Guanine Signals for DNA Biosensor Applications

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Abstract. This study was designed to investigate the performance of carbon nanotubes (CNT) modified carbon paste and carbon printed electrodes (SPE) produced in laboratory conditions. The effect of carbon nanotube use on signal enrichment was determined by using cyclic voltammetry (CV), square wave voltammetry (SWV) or differential pulse voltammetry (DPV) techniques based on potassium ferricyanide/ ferrocyanide or guanine signal. The application of different activation procedures to the electrode surface such as chemical (H_2SO_4, acetone, N,N-Dimethylformamide or NaOH) or electrochemical (different potential applications) were presented in this study. It was observed that the activation procedure applied to the nanotube modified electrode has strong effects on signal enrichment. From these procedures it was determined that the guanine signal obtained in activation with NaOH increased about 62-fold. It was also found that different nanotube species gave different responses to the activation processes. The optimum conditions of the nanotube-based biosensor were also presented.

***Keywords*:** Electrochemical techniques, DNA, biosensor, carbon nanotubes, screen printed electrode(SPE), Nucleic Acid Hybridization.

DNA Biyosensör Uygulamaları İçin Karbon Nanotüp Modifiye Yüzeylerin Ferrisiyanür ve Guanin Sinyallerine Dayalı Olarak Elektrokimyasal İncelenmesi

Özet. Bu çalışmada karbon nanotüpler (CNT) ile modifiye edilmiş karbon pastası (CPE) ve laboratuvar koşullarında basılarak üretilen perde baskılı karbon (SPE) elektrotların performansı karşılaştırılmış ve dönüşümlü voltametri(CV), kare dalga voltametri (SWV) veya diferansiyel puls voltametri (DPV) teknikleri kullanılarak elde edilen potasyum ferri / ferrosiyanür veya guanin sinyallerindeki artış miktarı tayin edilmiştir. Bu çalışmada elektrotlara kimyasal (H_2SO_4, aseton, N, N-Dimetilformamid veya NaOH) veya elektrokimyasal (farklı potansiyel uygulamaları) gibi farklı aktivasyon prosedürleri uygulanmıştır. Nanotüp modifiye elektrota uygulanan aktivasyon prosedürünün sinyal zenginleşmesi üzerine güçlü etkileri olduğu gözlenmiştir. Bu prosedürlerden NaOH ile aktivasyonda elde edilen guanin sinyalinin yaklaşık 62 kat arttığı tespit edilmiştir. Bu çalışmada ayrıca farklı nanotüp türlerinin aktivasyon proseslerine farklı yanıtlar verdiği de bulunmuştur. Nanotüp bazlı biyosensörün optimum şartları da ayrıca sunulmuştur.

***Anahtar Kelimeler*:** Elektrokimyasal teknikler, DNA, biyosensör, karbon nanotüpler, perde baskılı elektrot (SPE), Nükleik asit hibridizasyonu.

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1. INTRODUCTION

New generation DNA biosensor systems, including simple, precise, fast and easy-to-use methods, continue to be developed for routine medical applications such as genetic diagnosis along with the advancements in nanotechnology. These bio-devices designed for the specification of many genetic disorders include various nanomaterial-based assay methodologies. Among them electrochemical DNA biosensors, which were developed as an alternative to classical test methods and other biosensor systems, have some powerful benefits such as the monitoring of signals are more rapid and direct [1] than other i.e. optical or piezoelectric biosensor systems. However, in all cases, DNA detection with these nanosystems is usually based on signal enrichment. In fact, the aim of using nanomaterials in biosensor systems is to provide more biomaterial binding to the sensor surface. In this case, when the signal is enhanced, the limit of detection can be reduced considerably. Carbon nanotube(CNT) contained electrochemical biosensors have still received considerable interest from researchers because of their rapid electron transport and high biomolecule binding capacity via self-assemble strategies [2-6]. They have been used for many of detection purposes up to now such as drug-DNA interactions [7, 8], DNA hybridization sensing [9, 10], biomolecular interactions by hybrid assemblies [11-13] etc.

Hexagonal graphitic sheets-based CNTs are divided into two groups as single-walled carbon nanotubes (SWCNT) and multi-walled carbon nanotubes (MWCNT). They both have a very high surface area with nanometers range and their lengths can be micron levels. CNTs have still been used for preparation of a sensitive sensing layers in the field of biosensor design technologies due to amplifying recognition events. However, in order to increase the sensing signal, it is necessary to find some optimum conditions. Because the amount of signal increase, arising from the nanomaterial is influenced by experimental conditions as well as nanomaterial characteristics. For example, SWCNTs-modified electrode gave lover detection limit than MWCNTs modified one for the amperometric determination of hydrogen peroxide and phenolic compounds that was reported by Chekin et al. [14] Another example was presented by Erdem et al. related to CNTs-based biosensor design for the detection of Microcystis spp gene sequences. In their work, higher oxidation signals of guanine and adenine were obtained using MWCNTs-modified screen printed electrode in comparison to the SWCNTs-contained one[15]. Jeong et al. was developed the thrombin sensor by using bare(unmodified), gold nano particle (GNP), SWCNT or MWCNT modified electrodes in order to investigate the surface effect on SWV signals in that work [16].

On the other hand, in electrode systems carrying microchip substructure such as screen printed or pencil graphite electrodes, polymers are used in both commercial and hand-made electrodes to hold the powdery materials (i.e. gold particles, graphite, carbon nanotubes, etc.) together and to adhere them to the solid surface. This causes decrease in electron transfer kinetics and thus low signal acquisition from sensor surfaces. In electrochemical studies, application of the activation process to the electrode surface is very important in order to obtain reproducible results. The activation of the aforementioned disposable, nanomaterial-containing electrodes prior to use is also important for obtaining both reproducible and enriched signals. In addition, pretreatment of the electrodes helps to form -COOH groups on the SPE surface which increase the hydrophilicity of the surface and its reproducibility, thereby remove possible contaminants from the sensor [17]. In other words, there is an increment for active sites on the working electrode.

In this study, suitable experimental conditions for the use of nanotube modified electrodes in biosensor systems were investigated. It is aimed to obtain the highest electrochemical response with handmade carbon paste (CPE) and screen printed carbon electrode (SPE) containing bamboo or hollow type carbon nanotubes. As known, it is very important in electrochemical biosensor systems to reduce the detection limit of the sensor

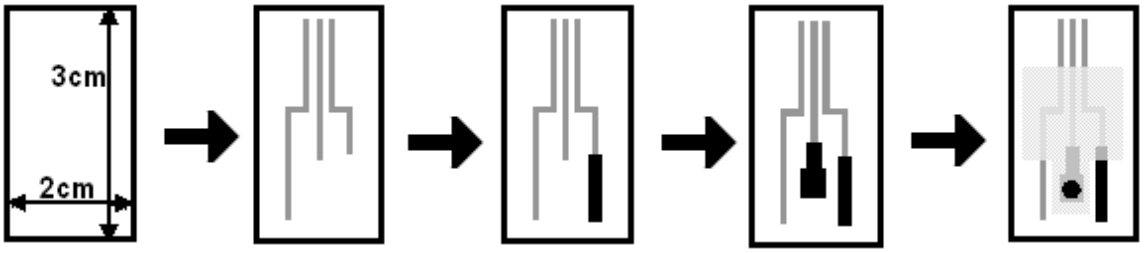
depending on the level of the signal obtained because of biochemical interaction. Here, the contribution of the nanomaterial to reducing the detection limit of the biosensor (or contribution to enhancing the signal obtained from the sensor) was explored using different experimental conditions. Basically, the disposable electrodes were prepared in the laboratory using a screen printed electrode machine to contain certain percentages of nanotubes firstly. Electrodes were then chemically or electrochemically activated by chemical treatment or applying positive potential onto the surface of the working electrodes in order to increase sensitivity and stability of the device. Electrochemical responses of potassium feri/ferrocyanide or guanine were measured and evaluated based on cyclic, square wave or differential puls voltammetry techniques. To our knowledge, optimum parameters (or operating conditions) of nanomaterial modified electrodes should be determined before the biosensor designs so that the developed biosensors can have features such as low detection limit, rapid analysis capability which are requirements of medical DNA tests. Advantages and disadvantages of these electrodes were evaluated in the following sections.

2. MATERIAL AND METHODS

2.1. Apparatus, Chemicals and Sample Preparation

AUTOLAB 12 potatiostat/galvanostat device (Eco Chemie, Netherlands) containing the software package GPES 4.9 (General Purpose Electrochemical System) was used for electrochemical measurements. Savitzky and Golay filter (level 2) of the software was preferred to smooth raw DPV voltammograms.

Disposable screen-printed electrodes (SPE) were prepared in the Firenze University, biosensor laboratory, Florence, Italy. They consisted of a silver (pseudo) reference electrode, a graphite working electrode (Ø = 3 mm) and a graphite auxiliary electrode as similarly reported in Carpini et al. [18] (Scheme 1). The carbon SPEs were printed by using a screen-printer machine (Model 248, DEK, Weimouth, UK). A Electrodag 423 SS model graphite ink and a Electrodag 410 PF model silver ink purchased from Acheson (Milan, Italy), a Vinyl fast 36-100 model insulating ink was obtained from Argon, Lodi, (Milan, Italy). An Autostat CT5 type polyester film purchased from Autotype (Milan, Italy) for the printing substrate. The silver ink was also used to serve as the conductive tracks. The thin sheet was fixed to the polycarbonate support so that the desired electrode thickness could be obtained. The SPE connector was used as the interface in all measurements.



Scheme 1. 2 cm wide and 3 cm long electrode printing process contains printing of silver ink for pseudo-reference electrode and electrical contact layers (a), printing of counter(b) and working(c) electrodes by using carbon ink, and printing of insulating layer(d) by using the insulating ink respectively. Carbon ink of the working electrode contained 1%, 2% or 5% ratio of carbon nanotubes.

Graphite powder and all types of CNTs (SWCNTs, hollow/bamboo structure of MWCNTs) purchased from Sigma-Aldrich.

The other three electrode system used in this study consisted of the surface-renewable carbon paste electrode (CPE) as the working electrode, a

reference electrode (Ag/AgCl), and a counter electrode (platinum wire). A 3 mm i.d. of glass tube was used as the body of the CPE and the mixture of carbon paste was packed in it. The electrical conductivity was provided using copper wire passed through the paste-filled tube. Carbon paste mixture composed of graphite powder (Fisher) and mineral oil (Acheson 38) in a 70:30 mass ratio. The CPE surface was polished by using a weighing paper before use.

2.1. Synthetic DNA materials

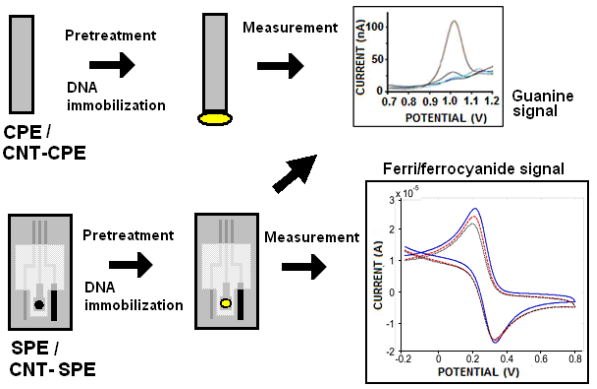
Double-stranded (ds) calf thymus DNA was purchased from Sigma-Aldrich (Milan, Italy). The stock solution of dsDNA (1000µg/ml) was prepared with Tris-EDTA buffer (10mM Tris–HCl, 1mM EDTA, pH 8.00) and store at freezer. More diluted solutions of DNA were prepared with 0.50M acetate buffer (pH 4.80) (ABS).

2.3. Chemicals

Sodium dihydrogen phosphate, disodium hydrogen phosphate, and acetic acid were obtained from Sigma (Milan, Italy). All reagents were of analytical grade and ultrapure water (18Ω) was used in all preparations of solutions (Elgastat, England). All experiments were carried out at room temperature provided by air condition (22.0-25.0 °C).

2.2. Methods

Scheme 2 shows the experimental details of the CNT-based biosensor. In the first part of the study, a 20µg/ml fish sperm double stranded DNA(dsDNA) was immobilized to various percentages such as 1%, 2%, 5% of SWCNT-containing CPE electrode. The increase in guanine oxidation signal was monitored by square wave and compared to the bare electrode. Guanine, which is an electroactive DNA base, is oxidized at about +1.0V potential at acetate buffer (pH:4.80) medium. In the second part, 20µg/ml dsDNA was attached to the surface of the SPEs that had been printed to contain SWCNTs/MWCNTs and the resulting of the electron transfer in the Nernst difussion layer was measured based on potassium ferri/ferrocyanide by cyclic voltammetry or guanine signal by differential pulse voltammetry. CNT-related signal enhancement was observed at different pretreatment conditions.



Scheme 2. Schematic diagrams illustrating the experimental steps of bare/CNTs-modified CPE and SPE electrodes and the detection of CNT-related signal enhancement based on guanine or ferri/ferrocyanide signals.

2.2.1. The preparation of sensor surfaces.

The pretreatment of SWCNTs-modified CPE was made by applying a potential of +1.70 V vs. reference electrode for 20s or 0 V(open circuit potential) for 20sec. in acetate buffer solution (0.50 M ABS; pH 4.80) without stirring. The 20µg/mL of dsDNA prepared in ABS was subsequently modified onto the activated surface of CPE by applying a fixed potential of +0.20 V for 5 min. The dsDNA immobilized CPE was then rinsed with ABS twice.

On the other hand, a 100 µL of ABS droplet was put on SWCNTs-contained SPEs for the pretreatment of their carbon working electrode surfaces. The different pretreatment and DNA immobilization conditions are as follows:

Table 1. The table showing the electrochemical activation processes applied in biosensor experiments with screen printed carbon electrode for guanine signal measurements.

Pretreatment (in ABS)	**DNA immobilization**
1.2V for 60s.	Passive adsorption for 2min.
1.6V for 60 s.	Passive adsorption for 2min.
1.8V for 60 s.	Passive adsorption for 2min.
1.6V, 120 s.+ 1.8V, 60 s.	Passive adsorption for 2min.
2.0V for 60 s.	Passive adsorption for 2min.
1.8V for 60 s.	+0.50 V for 120s. with stirring (200rpm.)

After pretreatment SPEs were then rinsed with 100 µL of blank ABS two times using micropipette. Each electrode strip was covered with a droplet including 20 µg/ml of dsDNA in ABS. After 2 min. of DNA immobilization under open circuit potential application (~0V), they were washed with the same buffer for 30s. with 200 rpm stirring. The electrodes were then coated with 100 µL of new drop of ABS so that their surfaces were not dry, and were left to stand until measurement.

2.2.2. Different pretreatment applications

Various activation techniques were applied to MWCNTs-modified SPEs (carbon ink contained 2% ratio of hollow or bamboo structure of MWCNTs) to achieve optimal signal enrichment. For example;

i. H_2SO_4 pretreatment: 2 CV scans (from -1.0 to +1.0V) in 0.2 M H_2SO_4
ii. Acetone pretreatment: 10 µL drop (acetone/water 1:1) on the working electrode surface for 10 min.
iii. N,N-Dimethylformamide pretreatment: 10 µL drop on the surface till dryness.
iv. NaOH pretreatment: +1.5V for 2min in 1M NaOH.

2.2.3. Electrochemical Measurements

The electrochemical transduction of the oxidation and reduction peaks of $Fe(CN)_6^{3-/4-}$ were recorded with cyclic voltammetry(CV) technique scanning from -0.25V to +0.65V potential range, 50mV scan rate and 3mV step potential. The oxidation signal of guanine was measured directly by square wave voltammetry scanning from +0.20 to +1.35V potential range versus Ag/AgCl reference electrode (or Ag-Pseudo-reference electrode) in the ABS aliquot at 40 mV pulse amplitude, 15mV step potential and 200 Hz frequency. The oxidation signal of guanine was also measured in blank ABS by DPV by scanning from +0.70 to +1.45 V with the amplitude of 50 mV at 16 mV/s scan rate. If necessary, the raw curve was treated by using the software program of GPES "Savitzky and Golay fitler" (level 2) with moving average baseline correction, using a "peak width" of 0.01 V.

Each procedure explained above was repeated at least three times and repetitive measurements were performed by refreshing the surface by using both electrochemical transducers.

3. RESULTS AND DISCUSSION

In theese studies, increased $Fe(CN)_6^{3-/4-}$ or guanine signals in the presence of CNTs were evaluated.

3.1. DNA detection based on guanine signal by using SWCNTs-modified CPE

First, the effect of the pretreatment applied to CPE surface on guanine signals was investigated and the results are shown in Figure 1. The peak currents of the guanine signal obtained from the unpretreated and pretreated electrodes by SWV technique are shown below. The oxidation of the guanine was obtained at about +1.00V in the absence and presence of SWCNTs modification.

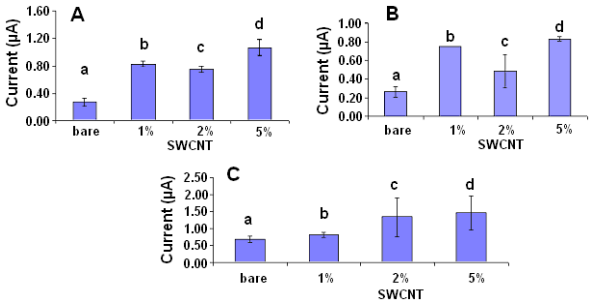


Figure 1. Graphs show the magnitude of guanine oxidation signals obtained from dsDNA immobilized surfaces (A) unpretreated CPE, (B) only 20s. ABS pretreated CPE (without potential application) (C) pretreated CPE applying at +1.7V potential during 20sec. and each column presents (a) bare electrode in the absence of carbon nanotube, (b) CNT-contained electrode in the presence of 1% SWCNTs, (c) 2% of SWCNTs and (d) 5% of SWCNTs modification onto CPEs.

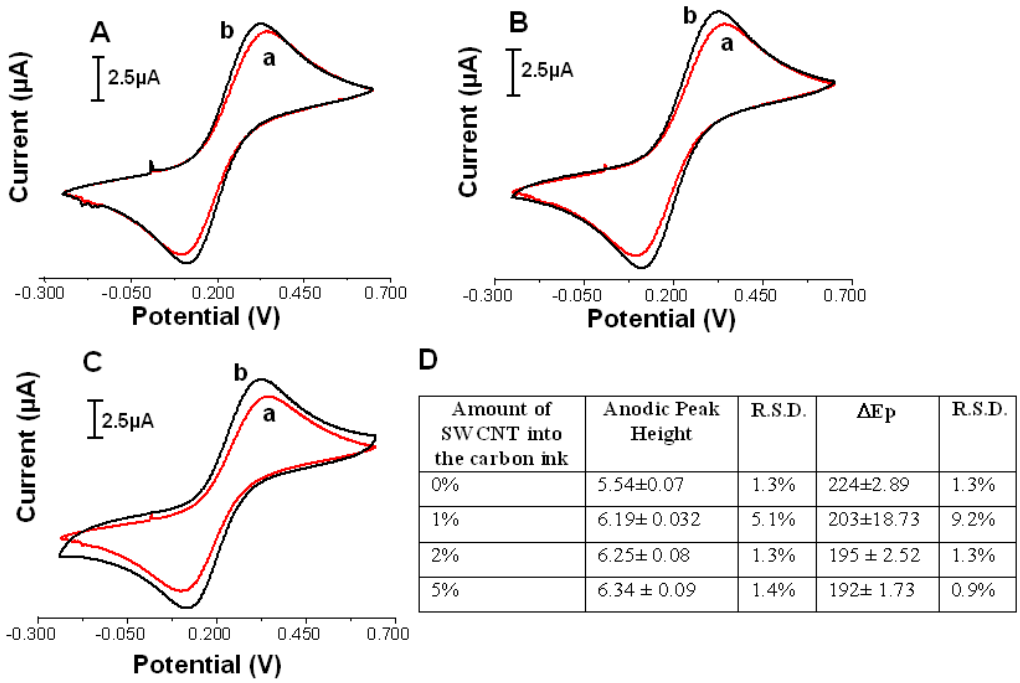
The magnitude of guanine peak observed with dsDNA modified unpreated (Figure 1A, a) and ABS pretreated (Figure 1B, a) CPEs in the absence of CNTs were about the same (main averege responses ~0.30µA). However, the electrochemically pretreated CPE (Figure 1C, a) gave the highest signal after dsDNA immobilization onto the CNTs-free electrode

surface. All of these results indicated that the activation process with potential application (+1.70V during 20sec.) enhances DNA binding onto the sensor surface.

On the other hand, guanine signal obtained from dsDNA modified SWCNTs electrodes were higher (Figure 1A; b,c and d, Figure 1B; b, c and d) than that obtained CNTs-free electrode (Figure 1A, a and Figure 1B, a) in the absence of potential application for pretreatment. When these increased signals are evaluated, it is seen that the electrode with different ratios of CNT has approximately 3-4 times more response than CNTs-free electrode (Figure 1A; b,c and d, Figure 1B; b, c and d). The highest guanine signal was obtained with 5% SWCNTs modified unpretreated electrode (Figure 1A; c). But when the pretreatment process is applied to the CNTs-contained electrode surface, only two fold signal enhancement was obtained from CPE with 2% and 5% SWCNTs without reproducibility.

A series of three repetitive DPV measurements of the guanine signal at 20 µg/ml concentration level of fish-sperm dsDNA modified unpretreated electrode with 5% SWCNTs resulted in reproducible results such as a mean response about 1.10µA with a relative standard deviation of 9.8% was calculated. As unsatisfactory and non-reproducible responses were obtained with DNA modified CPE, further studies were continued with SPE.

3.2. SWCNT-based studies by using SPE



Amount of SWCNT into the carbon ink	Anodic Peak Height	R.S.D.	ΔEp	R.S.D.
0%	5.54±0.07	1.3%	224±2.89	1.3%
1%	6.19± 0.032	5.1%	203±18.73	9.2%
2%	6.25± 0.08	1.3%	195 ± 2.52	1.3%
5%	6.34 ± 0.09	1.4%	192± 1.73	0.9%

Figure 2. Cyclic voltammogram of 10 mM $K_4[Fe(CN)_6]/K_3[Fe(CN)_6]$ (1 : 1) containing 1M KCl obtained from unpretreated bare (a) and SWCNTs-modified (b) SPE surfaces. The modification rates of SWCNTs in SPE are 1%(A), 2% (B) and 5%(C), respectively. The comparison of the electron transfer features of modified SPEs was also showed in the inset Table (D).

SWCNTs were simply mixed into the carbon ink of the SPE transducer with different rates as 1%, 2% and 5% as explained in the experimental part, and the electron transfer rate of modified electrodes was monitored using CV as shown in Figure 2. The differentiations in CV measurements were showed between the CNTs-free and CNTs-modified SPEs. According to the results, CNTs-contained electrode gave higher signal than bare electrode in each modification

conditions and the height of the anodic peak showed negative shift (approximately 21mV(1%), 29mV(2%) and 32mV (5%) respectively) in the Epa of $Fe(CN)6\ 3^{-}/4^{-}$ (Figure 2A, B and C). Figure 2D also presents anodic peak heights and ΔEp values of bare and modified electrodes with relative standard deviation values. The results indicated that the SWCNTs modified electrodes provide enhanced electron transfer rate (Figure 2A, B and C; approximately 11.7%, 12.8% and 14.4% of signal enhancement respectively) in comparison to the bare electrode. This result can be explained by carbon nanotube modified surfaces have better conductivity than simple carbon surfaces.

The effect of SWCNTs modification on intrinsic guanine response in different electrochemical pretreatment conditions by using SPE was showed in Figure 3.

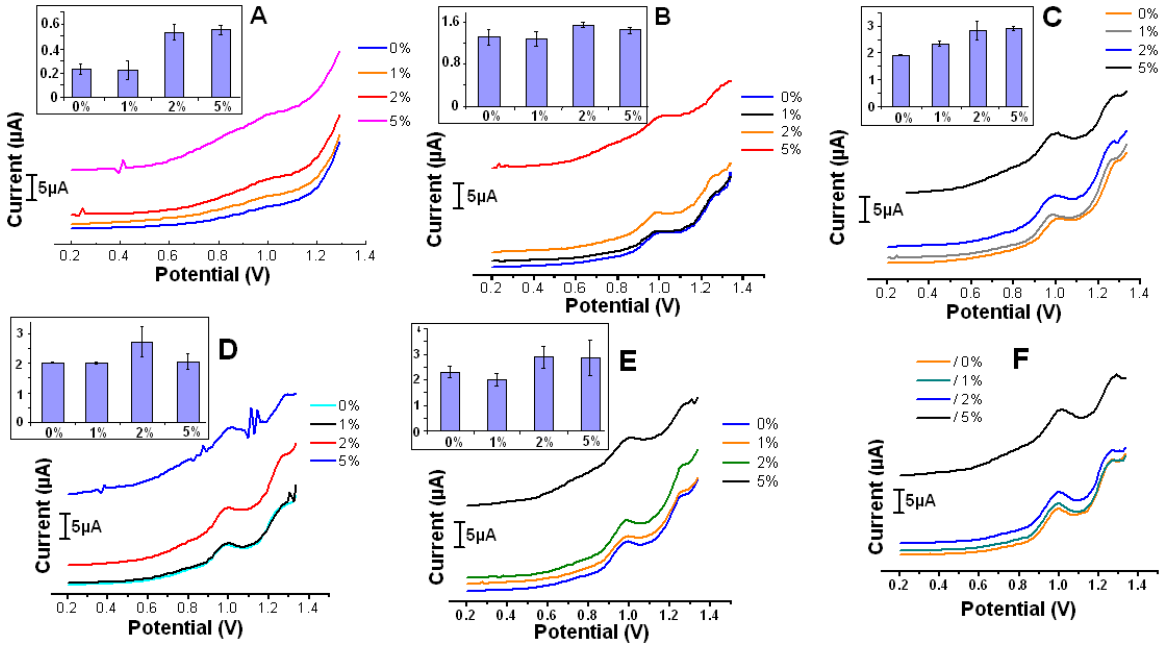


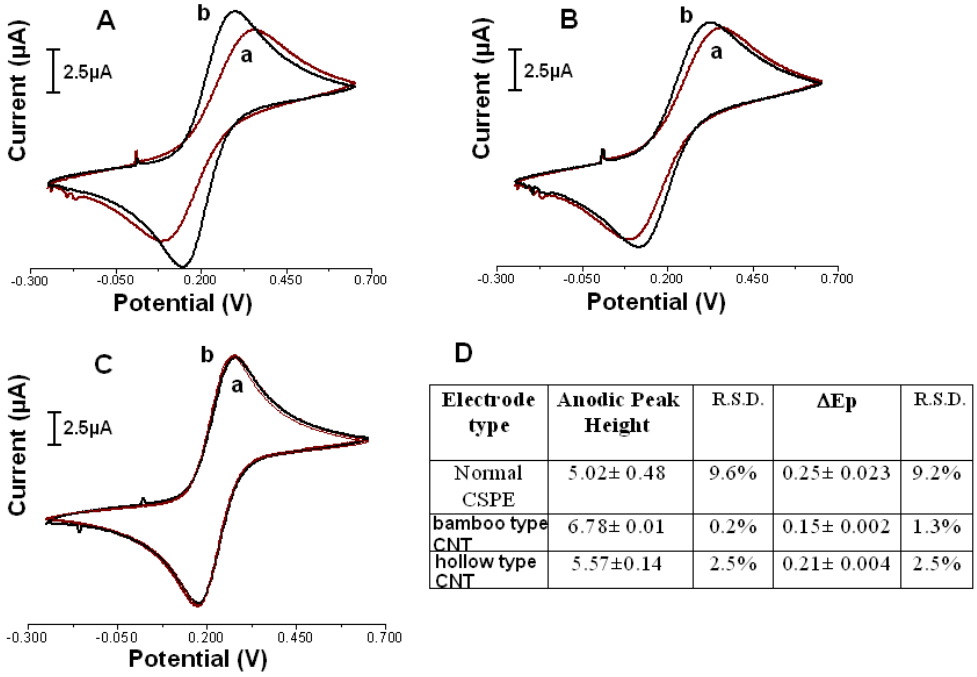
Figure 3. Voltammetric signal of guanine related to dsDNA modified sensor obtained by different pretreatment conditions such as (A) 1.2V for 60s.; (B) 1.6V for 60 s.; (C) 1.8V for 60 s.; (D) 1.6V, 120 s. and 1.8V, 60 s. together; (E) 2.0V for 60 s.; (F) 1.8V, 60s. respectively. dsDNA concentration was used as 20µg/mL and it was immobilized onto the surface of SPE by passive adsorption for 2 min. (A, B, C, D, and E) or potential application (+0.50 V for 120sec. with 200rpm. stirring, F). SWV parameters were described in the experimental section and Table 1.

The first thing to notice when looking at all the raw results obtained from figure 3A, B, C, D, E and F is that the baseline of 5% modification is higher than unmodified (0%) and other rate of CNTs modifications (1% or 2%). Another point is that the peaks obtained from the +1.2V potential applied electrodes for the pretreatment (Figure 3A, 0%, 1%, 2%, 5%) are much lower than the other electrochemically pretreated electrodes. (Figures 3B, C, D, E and F). However, the signal difference between bare and CNT-coated electrodes activated by applying the +1.2V potential (Figure 3A) is grater than more positive potential applied SPEs (Figure 3B, C, D, E and F). In Figure 3A, it is seen that approximately 0.23µA of guanine signals were obtained with both the unmodified (0%) and the modified electrode with 1% CNT. However, in the presence of 2% or 5% of CNTs modification onto SPE, about 2.4 times higher guanine signal (average response ~0.55µA) was measured in comparison to the unmodified or 1% ratio of CNTs modified sensor. The relative standard deviation (RSD) of three repetitive measurements was calculated as 10% in the presence of 2% CNTs modification and 7% in the presence of 5% CNTs modification onto the SPE. Acording to the Figure 3A, it is proved that more sensitive DNA analysis was achieved by using

CNTs-modified (2% or 5%) screen printed electrodes.

On the other hand, the expected increase at guanine signal could not be achieved with 1.6V potential applied CNT-contained electrodes (Figure 3B). In figure 3C, when compared the detection performance of modified electrodes with unmodified electrodes, it was observed that there was an approximately 1.5-fold increase in the presence of CNT (2% and 5% ratios). However, the reproducibility of the results was not good especially for 2% of CNTs modified transducer. When the pretreatment potential or time is further increased (Figure 3D and E) or if DNA immobilization is carried out under potential application (Figure 3F), it was observed that the CNTs modified and unmodified electrodes gave similar guanine responses (The average peak potential of all electrodes was obtained as 3-3.5µA). In other words, the signal increase expected with the modified electrodes could not be observed. This situation did not change with the extension of the DNA immobilization time (data not shown).

3.3. MWCNTs-based studies by using SPE.



Electrode type	Anodic Peak Height	R.S.D.	ΔEp	R.S.D.
Normal CSPE	5.02± 0.48	9.6%	0.25± 0.023	9.2%
bamboo type CNT	6.78± 0.01	0.2%	0.15± 0.002	1.3%
hollow type CNT	5.57±0.14	2.5%	0.21± 0.004	2.5%

Figure 4. Voltammograms obtained from bare (a) and MWCNTs-modified (b) SPE surfaces with the solution of 10 mM $K_4[Fe(CN)_6]/K_3[Fe(CN)_6]$ (1 : 1) containing 1M KCl. The modifications of 2% ratio of MWCNTs in SPE are bamboo structure (A and C), hollow structure (B) and the surface of the SPEs were unpretreated (A and B) or pretreated (C). CV measurement parameters as explained in Figure 2. The comparison of the electron transfer features of modified SPEs was also showed in the inset Table (D).

As explained in the experimental part, MWCNTs were simply mixed into the carbon ink of the SPE transducer with 2% ratio (In the direction of the previously obtained results from DNA-based studies, 2% CNT modification was performed on the electrodes.). Differentiations in CV measurements performed in $Fe(CN)_6^{3-/4-}$ solution were presented in Figure 4. In this experiment, CNTs-free and different types of MWCNTs-modified SPEs were used to to measure the effect of CNT modification on conductivity. Figure 4A and 4C show the responses obtained from bamboo-type MWCNTs modified SPE and Figure 4B presents the CV of hollow-type CNT-contained electrode. Red lines of each figure show the response of unmodified (CNTs-free) electrode. The most increase at the signals of ferri/ferro cyanide (~ 3.2 folds) was obtained with bamboo-structure MWCNTs modified and unpretreated SPE (Figure 4A, black line), in contrast to to the

signals observed using hollow-structure CNT modified (Figure 4C, black line) and pretreated bamboo-type CNT-modified SPEs (black line of Figure 4B). The electrochemical pretreatment (1.6V for 2min and 1.8V for 1min in acetate buffer) protocol for SPE destroyed the signal enhancement obtained by bamboo-type MWCNT modification (Figure 4C, black line). Since pretreatment was required for electrode surface optimization, it was decided to apply different pretreatment procedures in subsequent experiments. When all the results obtained using 2% bamboo-type MWCNT-modified SPEs were evaluated (Figure 4A and D), it was observed that these electrodes gave about 35% more $Fe(CN)_6^{3-/4-}$ signal than the bare electrode(Figure 4A, black line) and 22% more than the SPE containing SWCNT(Figure 2B and D) under the same experimental conditions.

On the other hand, if hollow-type MWCNT(Figure 4B and D) is used for the modification of SPE, only 11% increase in signal was observed compared to bare electrode. Thus, the high level increment at $Fe(CN)_6^{3-/4-}$ signal (Figure 4A) indicate that bamboo-type MWCNTs can provide enhanced electron transfer rate and higher surface area on the SPE. The comparison of the electron transfer features of modified SPEs based on ΔEp values were also showed in the Table (D).

Different pretreatment conditions

Different pretreatment procedures were applied [19-22] to the sensor surface to obtain the highest and reproducible responses from the CNT modified electrode. All these procedures were used for the analysis of different target molecules such as organophosphates [22].

Figure 5 shows the average signals of ferri/ferrocyanide obtained after the different pretreatment applications on MWCNT-contained SPE surface such as (A and B) H_2SO_4 pretreatment: 2 CV scans (from -1.0 to +1.0V) in 0.2 M H_2SO_4 for bamboo type CNT(A) and hollow-type CNT(B) modified electrodes; (C and D) Acetone pretreatment: 10 µL drop (acetone/water 1:1) on the working electrode surface for 10 min. for bamboo type CNT(C) and hollow-type CNT(D) modified electrodes; (E) N,N-Dimethylformamide pretreatment: 10 µL drop on the surface till dryness for bamboo-type CNT modified electrode; (F) NaOH pretreatment: +1.5V for 2min in 1M NaOH for bamboo-type CNT modified electrode respectively. CV parameters were described in the experimental section and Figure 2.

According to the Figure 5, when NaOH(1M) pretreatment was applied to the SPE by using +1.5V potential for 2min. or diluted acetone solution (1:1) was used for pretreatment of the SPE for 10 min duration better results were obtained with them when compared to the H_2SO_4 or dimethyl formamide activated electrode surfaces based on ferri/ferrocyanide signal.

The signal increment ratio about 70% was found to be the best result obtained with the acetone pretreated and bamboo-structure CNT modified SPE (Figure 5C). However, with other H_2SO_4 (Figure 5A) and NaOH(Figure 5F) pretreatment conditions, a signal increase of about 30% was obtained with the bamboo-structure CNT modified electrode.

On the other hand, no sufficient increase in DNA signals could be obtained with pretreatment procedures at acidic pH throughout the study. For this reason, further studies were carried out with acetone and NaOH-activated electrodes for label-free biosensor applications. The studies based on guanine signal measurement performed with dsDNA are shown in Figure 6.

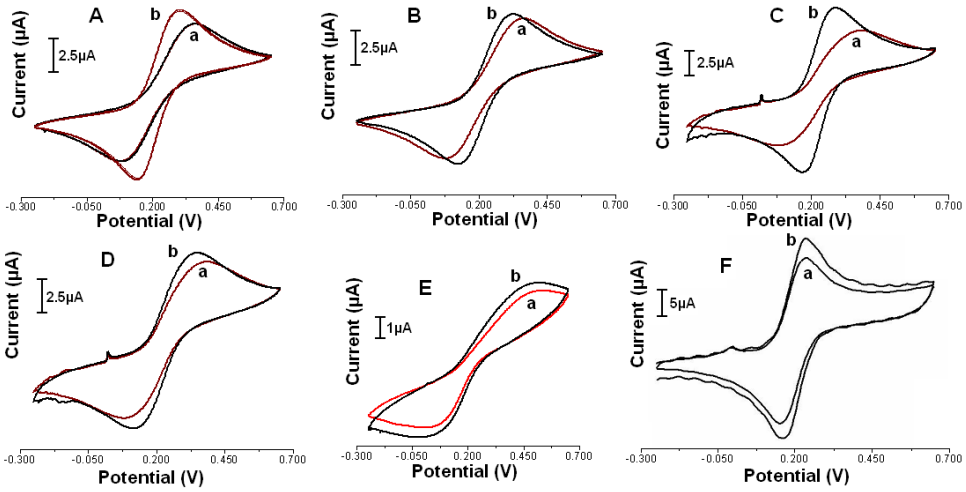


Figure 5. Voltammograms obtained from bare (a) and MWCNTs-contained (b) SPE surfaces with the solution of 10 mM $K_4[Fe(CN)_6]/K_3[Fe(CN)_6]$ (1: 1) containing 1M KCl. The modifications of 2% ratio of bamboo(A,C, E and F) or hollow-structure(B and D) MWCNTs in SPE the surface of the SPEs were pretreated with H_2SO_4 (A, B), acetone (C,D), Dimethylformamide(E) or NaOH (F). CV measurement parameters as similarly with the Figure 2.

SPEs were pretreated with H_2SO_4 (A, B), acetone (C,D), Dimethylformamide(E) or NaOH (F). CV measurement parameters as similarly with the Figure 2.

In label-free DNA biosensor studies it is expected that the guanine signal increase is obtained with the nanomaterial modified electrodes. For this purpose, 20 ppm dsDNA was immobilized in acetone(Figure 6A) or NaOH(Figure 6B) activated surfaces for 1 hour. After washing, guanine and adenine signals obtained from bare SPE or bamboo-structure CNT-modified SPE measured by DPV method were examined.

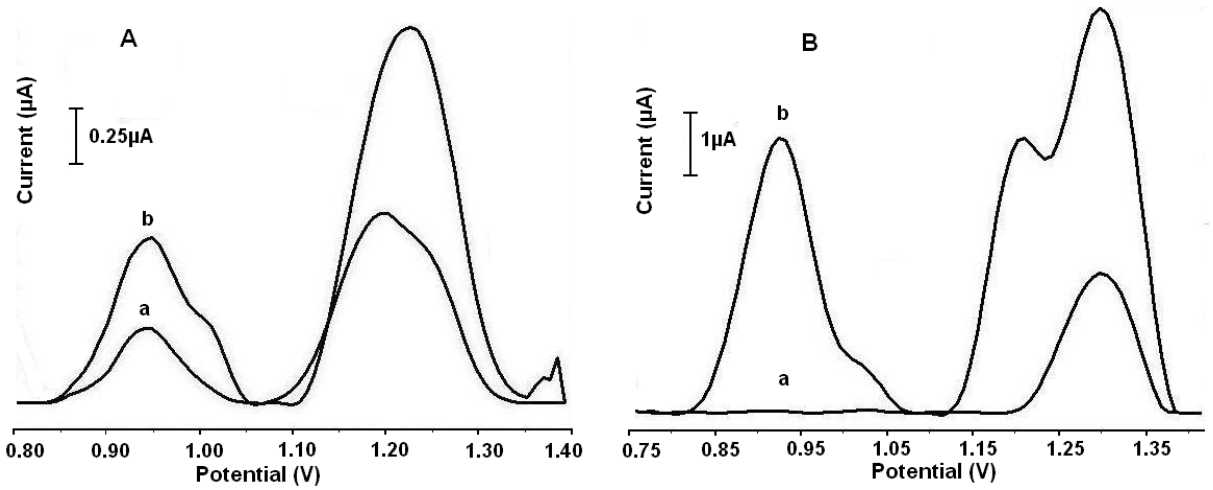


Figure 6. Influence of acetone (A) and NaOH(B)-based pretreatment process on the sensitivity of biosensor by using guanine-contained double stranded DNA. DPV of guanine oxidation signals obtained from DNA-modified bare (a) and bamboo-structured MWCNTs modified (b) electrodes.

The average guanine signal was observed as 332nA with bare electrode and 731nA with nanomaterial modified SPE by using acetone pretreatment method (Figure 6A). The CNT-modified electrode (Figure 6A,b) showed nearly two times increase at guanine response when compared to the bare electrode(Figure 6A,a). On the other hand, the average guanine response was

obtained as 4.36µA with NaOH-pretreated and CNT-contained SPE (Figure 6B,b). In comparison to the responses observed by using 20 µg/mL dsDNA immobilized bare electrode (Figure 6B,a), approximately 62 folds increment at the guanine peak was obtained by using DNA modified CNT-included SPE (Figure 6B, b). This huge signal enhancement originated from bamboo-type carbon nanotubes. The results of this experiment showed that carbon nanotubes could be used successfully to reduce the detection limit in biosensor design studies. On the other hand, in adenine signals obtained at about 1.25V(second peaks of Figure 6A and B at about 1.25V), significant signal increases were obtained with nanotube modified electrodes.

If the acetone and NaOH pretreatment techniques are compared in terms of ferricyanide/ferrocyanide signal increase between bare and CNT-modified electrode, acetone-based method showed 3.2 times more increase (Figure 5C) than NaOH pretreatment technique(Figure 5F). However, when the obtained guanine signals are evaluated, the signal obtained by the NaOH based pretreatment is much more than the acetone based method. This demonstrates the importance of pretreatment in biosensor studies. As a result, the guanine signal obtained after DNA immobilization on the electrode should be carefully examined after a certain increase in the electron transfer rate to be observed by the activation of the nanomaterial modified electrode.

There are also numerous conditions for pretreatment of SPEs in the literature for different research purposes [23]. For example, Morrin et al applied an electrochemical pretreatment based on CV method scanning between +1.2 V and +1.5 V in 0.2M H_2SO_4 solution [24]. Lee et al. also used CV technique scanning from -0.5V to +0.2V for 20 min in 0.1M $NaNO_3$[25.]. In another work, screen printed carbon electrodes were activated in saturated Na_2CO_3 solution at +1.2V for 5 min [17]. Patris et al. pretreated SPEs for 1.5 min in H_2SO_4 under +1.6V potential application [26]. When we compared our simple NaOH pretreatment protocol with these studies, it has advantages such as shorter activation time (2 min) and potential application (+1.5 V). In addition, it is the first time that NaOH pretreatment method and bamboo-type CNTs modification have been shown to increase the DNA signal by nearly 62 times.

4. CONCLUSION

The bamboo-type MWCNTs structure was modified to the SPE surface which is suitable for measuring intrinsic guanine oxidation signal directly. The main advantage of the applied protocol is its effective pretreatment step applied on CNTs-contained SPE providing highly enhanced signal with high sensitivity. The effect of bamboo-type carbon nanotubes on guanine signal enrichment was shown for the first time in this study. The modification of SPEs and its pretreatment with NaOH provide enhanced adsorption of DNA on the surface of the sensor, and thus they offer nearly 62 times increased sensitivity besides low detection limit when compared with CNTs-free sensor. In the new biosensor systems to be designed, if the method in this sensor based on label-free measurement is used, a new and strong alternative will be introduced to the traditional methods. Because indicator-free detection is greatly simplify the sensing methodology by eliminating the use of label and the need for the additional time-consuming process for indicator-DNA interaction. Future plan for this laboratory will be the development of hybridization-based DNA biosensors that can be converted into microchips.

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